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Development of Reversed-Polarity CE/ESI/MS as a New Screening Tool for DNA Adducts

David M. Chambers, Ken Turteltaub, Bob Mauthe and Brian Andresen
Lawrence Livermore National Laboratory, Livermore CA 94551

Covalently bound DNA adducts, which are believed to promote mutagenesis and carcinogenesis, are generally formed when an electrophilic chemical reacts with nucleophilic sites located on DNA bases. These adduct-forming chemicals can be introduced through diet, lifestyle choices (e.g., smoking), pollution, and exposure in the workplace. Unfortunately, monitoring the formation of DNA adducts to identify exposure is difficult because relevant levels yield on the order of one adduct per 10E6 to 10E8 nucleotides. At these low levels detection must exceed low pmole/mg of DNA, which is already a minor cellular component of approximately 0.1% by weight. Although a number of highly sensitive techniques such as immunoassays, fluorescence and radioactive postlabeling achieve these low levels of detection, they provide limited structural information needed for structural identification. With recent advances in the development of atmospheric pressure ionization mass spectrometric techniques a new class of methods is emerging that provide both the sensitivity and specificity suitable for adduct analysis.

Capillary electrophoresis/electrospray/mass spectrometry (CE/ESI/MS) is one approach being developed in our laboratory for this application. In this work nucleotide monomers prepared from cell extracts are separated by reversed polarity CE and ionized by negative ESI. Apart from its characteristically high separation efficiency and speed, CE is attractive for isolating the nucleotide adduct from other monomers because it offers relative freedom from capillary clogging and increased selectivity through electrokinetic injection, both helpful when sampling a complex mixture. Although it is less routine, we have found that negative ESI yields a relatively clean spectral background and an abundant M^- signal. The significance of capillary coatings as well as the selection of CE buffers and organic modifiers necessary to maintain high separation efficiency and at the same time a stable and efficient negative ESI are described. Validation of this approach is performed using 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine, a potent mammalian cell mutagen and carcinogen found in cooked meats, which has been under investigation at LLNL in relation to human exposure and risk assessment.

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